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**DATA SUMMARY & TEST PLAN**  
**For**  
**2-Hydroxy-4-n-Octoxybenzophenone**

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October 1, 2001

OVERVIEW

Cytec Industries Inc. and Ciba Specialty Chemicals Corporation hereby submit for review a test plan for 2-Hydroxy-4-n-Octoxybenzophenone under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of these companies to use existing data to adequately fulfill the Screening Information Set (SIDS) for environmental fate endpoints, ecotoxicity tests, and human health effects for this substance. We believe that adequate data exist to fulfill all the requirements of the HPV program without the need for additional testing.

US EPA High Production Volume (HPV)  
Chemical Challenge Program

**Data Summary  
&  
Test Plan**

**2-Hydroxy-4-n-Octoxybenzophenone**  
**CAS No. 1843-05-6**

October 1, 2001

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## DATA SUMMARY TABLE

CAS No. 1843-05-6	DATE	RESULTS	FULFILLS REQUIREMENT
<b>PHYSICAL/CHEMICAL ELEMENTS</b>			
Melting Point	2000	48 -49 °C	Yes
Boiling Point	1968	> 300 °C (Decomposes)	Not applicable
Vapour Pressure		--	Not applicable
Partition Coefficient	2000	Log Kow > 6.00	Yes
Water Solubility	2001	<7.3 x 10 <sup>-7</sup> g/L of solution at 20.0±0.5°C	Yes
<b>ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS</b>			
Photodegradation		For reaction with hydroxyl radical, predicted rate constant = 218.14 x 10 <sup>-12</sup> cm <sup>3</sup> /molecule-sec predicted half-life = 0.59 h [EPIWIN Program]	Yes
Stability in Water	2001	The estimated half-life at pH 4, 7, and 9 at 25°C is > 1 year.	Yes
Fugacity	2000	Predicted distribution using Level III Fugacity model Air 0.09% Water 8.2% Soil 29.5% Sediment 62.2% [EPIWIN Program]	Yes
Biodegradation	1989	Not Biodegradable	Yes
<b>ECOTOXICITY ELEMENTS</b>			
Acute Toxicity to Fish	1988	Zebra fish (Brachydanio rerio): LC <sub>50</sub> (96 h) > 100 mg/L	Yes
Toxicity to Aquatic Plants	1992	Green Algae (Scenedesmus subspicatus) EC <sub>50</sub> (0 -72 h) > 100 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1988	Daphnia magna Straus 1820 EC <sub>0</sub> (24 h) > 10 mg/L EC <sub>50</sub> (24 h) > 52 mg/L	Yes
<b>MAMMALIAN TOXICITY</b>			
Acute Toxicity	1965	>10 g/kg (rats)	Yes
Genetic Toxicity: Gene Mutations	1991	Non-mutagenic to bacterial cells	Yes
Genetic Toxicity: Chromosomal Aberration	2001	Non-clastogenic to human lymphocytes <i>in vitro</i>	Yes
Repeated Dose Toxicity	1965	Rat 30-day Dietary	Yes
	1965	Rat 90-day Dietary NOEL = 0.6 % (6000 ppm)	Yes
	1968	Rat 90-day Dietary NOEL = 0.15 % (1500 ppm)	Yes
	1969	Rats 90-day Dietary NOEL = 1000 ppm	Yes
Reproductive/ Developmental Toxicity	1969	Rats NOEL = 0.6% (6000 ppm) for 4 Successive Generations	Yes

**PROPOSED TEST PLAN**

CAS# 1843-05-6	Data Available	Data Acceptable	Testing Required
Study	Y/N	Y/N	Y/N
<b>Physical/Chemical Characteristics</b>			
Melting Point	Y	Y	N
Boiling Point	NA	NA	N
Vapor Pressure	NA	NA	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
<b>Environmental Fate</b>			
Photodegradation	Y	Y	N
Hydrolysis	Y	Y	N
Fugacity	Y	Y	N
Biodegradation	Y	Y	N
<b>Ecotoxicity</b>			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y	Y	N
Acute Toxicity to Algae	Y	Y	N
<b>Mammalian Toxicity</b>			
Acute Toxicity	Y	Y	N
Repeat Dose Toxicity	Y	Y	N
Genetic Toxicity: Gene Mutations	Y	Y	N
Genetic Toxicity: Chromosomal Aberration	Y	Y	N
Developmental Toxicity	Y	Y	N
Reproductive Toxicity	Y	Y	N

## GENERAL INFORMATION

### A. INTRODUCTION

On November 22 and 29, 1999, respectively, Cytec Industries Inc. (Cytec) and Ciba Specialty Chemicals Corporation (Ciba) agreed to participate in the Environmental Protection Agency's (EPA) High Production Volume Chemical Challenge Program. By participating in this program, Cytec and Ciba agreed to assess the adequacy of existing data, design and submit test plans to fill data gaps where necessary and appropriate, provide test results, and prepare summaries of the data characterizing each chemical sponsored.

The sponsored chemical addressed in this test plan is 2-hydroxy-4-n-octoxybenzophenone, (CAS # 1843-05-6).

### B. GENERAL SUBSTANCE INFORMATION

Chemical Name: 2-hydroxy-4-n-octoxybenzophenone

Description: The substance is a pale, cream-to-white powder with friable lumps.

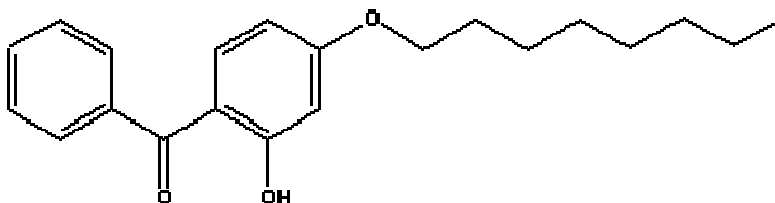
Chemical Abstract Service Registry Number: CAS # 1843-05-6

Common Name: Benzophenone-12

Chemical Formula:  $C_{21}H_{26}O_3$

Molecular Weight: 326.42

Structure:



### C. GENERAL USE INFORMATION

2-Hydroxy-4-n-octoxybenzophenone is an effective photostabilizer for a variety of plastic systems. It may be used in food packaging materials as an antioxidant and stabilizer and in addition may be used as a stabilizer in petroleum wax. As such, it is cleared under the 21 CFR (Code of Federal Regulations) §178.2010 for use as a stabilizer in polypropylene, polyethylene, olefin copolymers, and poly(methylpentene) complying with limitations set forth in §177.1520c and also under 21 CFR §178.3710. When used in packaging materials, 2-hydroxy-4-n-octoxybenzophenone prevents UV-radiation from reaching the stored product and increases the stability of the container. In order to be approved by the FDA for such uses, 2-hydroxy-4-n-octoxybenzophenone has been thoroughly evaluated for its potential toxicity.

**DATA SUMMARIES****1. MELTING POINT**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	Directive 84/449/EEC, A.1 "Melting point/ melting range" From Material Safety Data Sheet <sup>1</sup>
GLP:	Yes
Year:	1993
Results:	48 - 49 °C
Remarks:	The melting point was obtained from MSDS of Ciba Specialty Chemicals Corporation and is consistent with that on the MSDS of Cytec Industries Inc. The method of determination and other details were not reported. The melting point estimate is assigned a reliability code of 2g <sup>2</sup> (data from handbook or collection of data).
References:	<sup>1</sup> Ciba Specialty Chemicals Corp.  <sup>2</sup> See listing of codes, p.35.

## 2. BOILING POINT

Test substance: 2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6

Not applicable. This substance is a solid and decomposes at temperatures >300 °C<sup>1</sup>

References: <sup>1</sup>Patel, Y.M. Levinskas, G.J., and Shafer, C.B. 1968. Toxicity and Metabolism of 2-Hydroxy-4-n-Octoxybenzophenone. *Fd Cosmet. Toxicol.* Vol. 6, pp. 199-208.



### 3. VAPOR PRESSURE

Test substance:

2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6

Not applicable. This substance is a solid

**4. PARTITION COEFFICIENT**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	From Material Safety Data Sheet <sup>1</sup>
GLP:	Yes
Year:	2000
Results:	Log Kow > 6.00
Remarks:	The partition coefficient was obtained from the MSDS of Ciba Specialty Chemicals Corporation. The method of determination and other details were not reported. The partition coefficient estimate by this method is assigned a reliability code of 2g <sup>3</sup> (data from handbook or collection of data). The partition coefficient was also estimated by KOWWIN Program (V1.66) <sup>2</sup> . The calculated value is 6.96.
References:	<sup>1</sup> Ciba Specialty Chemicals MSDS  <sup>2</sup> Syracuse Research Corporation, Syracuse, NY. Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998  <sup>3</sup> See listing of codes, p.35.

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Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	Estimated using TOPKAT v.3.05
GLP:	Does not apply to estimated data based on Structure Activity Relationship
Year:	1999
Results:	Log Kow = 6.416
Remarks:	The partition coefficient estimated by this method is assigned a reliability code of 2f <sup>2</sup> .
References:	<sup>1</sup> Health Designs, Inc. Rochester, NY. 1999.  <sup>2</sup> See listing of codes, p.35.

## 5. WATER SOLUBILITY

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	Column Elution Method, Method A6 of Commission Directive 92/69/EEC and OECD Guideline Method 105
Temperature:	20.0 ± 0.5°C
GLP:	Yes
Year:	2001
Results:	<7.3 x 10 <sup>-7</sup> g/L of solution
Remarks:	<p><u>Preliminary Test:</u> An aliquot (0.300 g) of test material was diluted to 900 ml with glass double-distilled water. After shaking at 30°C for 68 hours and standing at 20°C for 24 hours, the solution was centrifuged, filtered and analysed. The preliminary estimate of water solubility was &lt;6.44 x 10<sup>-5</sup> g/L.</p> <p><u>Definitive Test:</u> An aliquot (0.1051 g) of test material was dissolved in acetone (20 ml). Glass beads were added and the solvent removed using a rotary evaporator. The elution apparatus was set up consisting of glass micro-column fitted with a plug of glass wool, and connected to a recirculating pump and a reservoir capable of holding approximately 2 L of water. The circulating water was maintained at 20.0 ± 0.5°C by means of a water bath. The whole system was flushed for 2 hours, and the water was discarded. The reservoir was re-filled with fresh glass double-distilled water and the coated bead loaded into the micro-column. After allowing the coated beads to soak for ~15 hours, the re-circulating pump was switched on and the first 25 ml of eluate discarded. Aliquots (~250 ml) of sample solution were taken from the column at intervals of at least ten bed volumes of eluate and centrifuged. This procedure was carried out in duplicate. Analysis of the test material in the sample solutions was determined by high performance liquid chromatography (HPLC).</p> <p>The water solubility determination by this method is assigned a reliability code of 1a<sup>3</sup>.</p>
References:	<p><sup>1</sup>SafePharm Laboratories Limited. Determination of Abiotic Degradation, Hydrolysis as a function of pH and Water Solubility. SPL Project Number: 971/121. Sponsored by Cytec Industries Inc., 2001.</p> <p><sup>2</sup> See listing of codes, p.35.</p>

## 6. PHOTODEGRADATION

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	Estimated by the AOP program (v. 1.90), <sup>1</sup> which estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2000
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical is rapid.  Rate constant: $218.14 \times 10^{-12} \text{ cm}^3/\text{molecule-sec}$ Half-life: 0.59 h
Remarks:	The photodegradation calculation by an accepted method is assigned a reliability code of 2f <sup>2</sup> .
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY. Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.  <sup>2</sup> See listing of codes, p.35.

## 7. STABILITY IN WATER

Test substance: 2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6

Method: Method C7 of Commission Directive 92/69/EEC and OECD Guidelines Method 111

GLP: Yes

Year: 2001

Results: The estimated half-life of the test material at 25°C is shown in the table below:

pH	Estimated half-life at 25°C
4	> 1 year
7	> 1 year
9	> 1 year

Remarks:

Preparation of buffer solutions:

The buffer solutions were filtered through a 0.2 mm membrane filter to ensure they were sterile before commencement of the test. Also, these solutions were subjected to ultrasonication and degassing with nitrogen to minimize dissolved oxygen content.

Buffer pH	Components	Concentration (mol dm <sup>-3</sup> )
4	Potassium hydrogen phthalate	0.01
7	Disodium hydrogen othrophosphate (anhydrous) Potassium dihydrogen othrophosphate Sodium chloride	6.00 x 10 <sup>-3</sup> 4.00 X 10 <sup>-3</sup> 4. 00 X 10 <sup>-3</sup>
9	Disodium tetraborate Sodium chloride	2.00 X 10 <sup>-3</sup> 4.00 X 10 <sup>-3</sup>

Preparation of samples:

Sample solutions were prepared in stoppered glass flasks at a nominal concentration of 3.44 x 10<sup>-7</sup> g/l in the three buffer solutions. Solutions were shielded from light and maintained at the test temperature.

Results:

Sample solutions at pH 4, 7, and 9 were maintained at 50.0 ± 0.5°C for 5 days. Aliquots of sample solutions were taken at various times and pH recorded. The concentration of the sample solution was determined by HPLC.

Less than 10% hydrolysis after 5 days at 50°C, equivalent to a half-life greater than 1 year at 25°C was the result for each of the buffer solutions tested.

The Abiotic Degradation, Hydrolysis as a function of pH determination by this method is assigned a reliability code of 1a<sup>3</sup>.

References:

<sup>1</sup>SafePharm Laboratories Limited. Determination of Abiotic Degradation, Hydrolysis as a function of pH and Water Solubility. SPL Project Number: 971/121. Sponsored by Cytec Industries Inc., 2001.

<sup>2</sup> See listing of codes, p.35.

**8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Test substance: 2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6

Method: EPI WIN level III Fugacity model.<sup>1</sup>

Year: 2000

GLP: No

Results: Distribution using level III fugacity model

Air	0.09 %
Water	8.2%
Soil	29.5 %
Sediment	62.2%

Remarks: The fugacity calculation by an accepted method is assigned a code of 2f<sup>2</sup>.

References: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY. Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>2</sup> See listing of codes, p.35.

## 9. BIODEGRADATION

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Batch No. EN 130125.82
Method:	The protocol generally followed OECD Guideline 301 B, algae growth inhibition test. Instead of centrifuged sludge, settled sludge was used. The substance was not dissolved but distributed in the test medium. The sludge concentration in the test bottle was reduced from 3.0 L to 1.5 L.
Species:	Bacteria collected from activated sludge of the sewage treatment plant.
Duration:	28 days
Temperature:	22 ± 2° C
Reference Substance:	Aniline MERCK No.: 1261
Concentrations:	Reference substance: 20 mg/L Test substance: 10.7 mg/ L, and 20.2 mg/ L.
GLP:	No
Year:	1989
Results:	The observed biodegradation was:

<i>Test Material</i>	<i>Dose</i>	<i>Result (in 28 days)</i>
reference substance	20 mg/L	84.3 %
test substance	10.7 mg/L	6 %
test substance	20.2 mg/L	5 %

The test substance is not biodegradable under these experimental conditions.

Remarks:	This study is assigned a reliability code of 2c <sup>2</sup> (comparable to guideline study with acceptable restrictions) according the criteria established by Klimisch <i>et al</i> (1997).
Reference:	<sup>1</sup> Report On The Test For Ready Biodegradability Of TK 10050 In The Modified Strum Test; Project No: 188 45 60; U. Bader, Dr. A. De Morsier; Ciba-Geigy Ltd. Basle, Switzerland.

<sup>2</sup> See listing of codes, p.35.



**10. ACUTE TOXICITY TO FISH**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Batch No. EN 130125.82
Method:	OECD Guideline No. 203 (Paris 1984).
Species:	Zebra Fish ( <i>Brachydanio rerio</i> )
Supplier:	West-Aquarium, D-3422 Bad Lauterberg
Length:	26 mm (22-29 mm)
Weight:	0.17 g (0.12 - 0.23 g)
Loading:	0.11 g/L
Test Concentrations:	10, 18, 32, 58, 100 mg/L (nominal)
Controls:	Blank: Water Vehicle: 100 mg DMF and 0.8 mg alkylphenol-polyglykol-ether per liter water
Exposure period:	96 h
Analytical monitoring:	No
GLP:	No
Year:	1988
Results:	LC <sub>50</sub> (96 h): > 100 mg/L LC <sub>0</sub> (96 h): > 100 mg/L Mortalities in blank and in vehicle: 0%
Remarks:	This study is assigned a reliability code of 2b <sup>2</sup> (guideline study with acceptable restrictions) according the criteria established by Klimisch <i>et al</i> (1997), as it was conducted under OECD Guidelines.
Reference:	<sup>1</sup> Report Test For Acute Toxicity Of TK 10050 To Zebra Fish; Project No.: 884561; Drs H. Rufli, A. De Morsier; Ciba-Geigy Ltd. Basle, Switzerland.

<sup>2</sup> See listing of codes, p.35.

## 11. TOXICITY TO AQUATIC PLANTS

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Batch No. EN 253644.02
Method:	According to the guidelines 87/302/EEC Algal growth inhibition test. The static <i>Scenedesmus subspicatus</i> toxicity screen was conducted in 100-mL Erlenmeyer flasks containing 50 mL of algae nutrient media or test solution. The temperature was maintained at $24 \pm 1^{\circ}\text{C}$ with continuous illumination with cold white fluorescent light. Each test concentration was tested in 3 replicates and the blank control in 6. Cell densities were measured at 24, 48 and 72 hours exposure.
Species:	Green Algae ( <i>Scenedesmus subspicatus</i> )
Initial cell density	10400 cells/mL
Test concentrations :	Test substance: 1.23, 3.7, 11, 33, and 100 mg/L (Nominal) Controls: Blank: Water Vehicle: 96.0 mg n-methyl-2-pyrrolidone and 4.0 mg polyoxy-ethylene-sorbitan-monooleate (TWEEN 80)/l
Exposure period:	72 h
Analytical monitoring:	No
GLP:	No
Year:	1992
Results:	EbC <sub>50</sub> (0-72 h): > 100 mg/L (growth inhibition) NOEbC <sub>50</sub> (0-72 h): 100 mg/L
Remarks:	This study is assigned a reliability code of 2c <sup>2</sup> (comparable to guideline study with acceptable restrictions) according the criteria established by Klimisch <i>et al</i> (1997).
Reference:	<sup>1</sup> Report On The Inhibition Test Of Chimassorb 81 to green algae ( <i>Scenedesmus subspicatus</i> ); Dr. A. Von Schulthess, Ciba-Geigy Ltd., Additives Division, CH-4002, Basel, Switzerland.

<sup>2</sup> See listing of codes, p.35.

**12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Batch No. 130125.82
Method:	OECD Guideline No. 202 (Part I, 1984).
Species:	Daphnia magna Straus 1820
Exposure period:	24 hours
Test Concentration:	0.58, 1.0, 1.8, 3.2, 5.8, 10, 18, 32, 58 mg/L (nominal)
Controls:	Vehicle: 94.5 mg 1-methyl-2-pyrrolidon and 0.6 mg alkylphenol-polyglycol ether per liter water Blank: Water.
Analytical monitoring:	No.
GLP:	No.
Year:	1988
Results:	EC <sub>50</sub> (24 h) calculated: 52 mg/L (confidential limits: 36-100 mg/L)  EC <sub>0</sub> (24h): 10 mg/L  Immobilization in blank 0 % Immobilization in vehicle 0 %
Remarks:	The study is assigned a reliability code of 2c <sup>2</sup> (comparable to guideline study with acceptance restrictions). Values are based on nominal concentrations.
Reference:	<sup>1</sup> Report On The Test For Acute Toxicity of TK 10050 To Daphnia Magna; OECE-Guideline No. 202, Part I, 1984; Project No.: 884562; Drs. A. de Morsier, H. Rufli; CIBA-GEIGY Ltd., Basel, Switzerland.

<sup>2</sup>See listing of codes, p.35.

### 13. ACUTE TOXICITY

#### Acute Rat Oral LD50

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	Test material dosed as a 20% aqueous dispersion.
Species/strain:	Carworth Farm Nelson strain Albinos Rats
Sex:	Male
No. Animals/Group:	10
Doses:	A single 10 g/kg dose
Post dosing observation period:	Dosing was followed by a 7-day observation period. No signs of intoxication were observed post-dosing. In addition, no abnormal findings were noted following gross autopsy.
GLP:	Conducted prior to GLP.
Year:	1965
Results:	<b>Oral LD<sub>50</sub> (rat) &gt; 10 g/kg.</b> 10 male CF Nelson albino rats were dosed with a 20% aqueous dispersion of the product at a dose of 10.0 g/kg. 0/10 died, and there were no signs of intoxication; the gross autopsy was normal.
Remarks:	This study is assigned a rating code of 2e <sup>2</sup> (meets generally accepted scientific standards, well documented and accepted for assessment).
References:	<sup>1</sup> American Cyanamid Company, Wayne, New Jersey. Report 65-58. June 30, 1965.

<sup>2</sup>See listing of codes, p.35.

## 14. GENETIC TOXICITY

### A. Ames Salmonella Mutagenicity Assay(1)

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Batch No. EN 254644.02
Method:	This study was not conducted under OECD guidelines, but was conducted using the methods described by Ames <i>et al</i> (1973, 1975). <sup>2,3,4</sup> The mutagenicity test was performed with the Escherichia coli strains TA 98, TA 100, TA 1535 and TA 1537 with and without microsomal activation according to Standard Operating Procedures of Genetic Toxicology,
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
GLP:	No
Year:	1991
Results:	In the experiments performed with and without microsomal activation, comparison of the number of histidine-prototrophic mutants in the controls and after treatment with TK 10050 revealed no marked differences.
Remarks:	This study is assigned a rating code of 2e <sup>5</sup> (meets generally accepted scientific standards, well documented and accepted for assessment).
References:	<sup>1</sup> Bacterial Mutagenicity Screening Test; CIBA-GEIGY Limited, Basel, Switzerland; September 02, 1991.  <sup>2</sup> Ames, B.N., Lee, F.D., and Durston, W.E., "An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. USA, 70, 782-786, 1973.  <sup>3</sup> Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D., "Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection," Proc. Natl. Acad. Sci. USA, 70, 2281-2285, 1973.  <sup>4</sup> Ames, B.N., McCann, J., and Yamasaki, E., "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364, 1975.  <sup>5</sup> See listing of codes, p.35.

**B. Ames Salmonella Mutagenicity Assay (2)**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6 Lot # W-8720
Method:	This study was not conducted under OECD guidelines, but was conducted using the methods described by Ames <i>et al</i> (1973, 1975). <sup>2,3,4</sup> The mutagenicity test was performed with the strains Salmonella typhimurium TA 98, TA 100, TA 1535 and TA 1537, and Escherichia coli WP-2 uvrA- with and without microsomal activation according to Standard Operating Procedures of Genetic Toxicology.
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537; Escherichia coli WP-2 uvrA-
GLP:	No
Year:	1981
Results:	In the experiments performed with and without microsomal activation, no mutagenic activity was noted at doses up to 1000 µg/plate. Doses tested were 100, 333, 1000, 2500, and 5000 µg/plate. Precipitate on plates was noted at 2500 and 5000 µg/plate doses. Background lawns appeared intact.
Remarks:	This study is assigned a rating code of 2e <sup>5</sup> (meets generally accepted scientific standards, well documented and accepted for assessment).
References:	<sup>1</sup> Bacterial Mutagenicity Screening Test; American Cyanamid Company, Report M81-28. February 6, 1981.  <sup>2</sup> Ames, B.N., Lee, F.D., and Durston, W.E., "An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. USA, 70, 782-786, 1973.  <sup>3</sup> Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D., "Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection," Proc. Natl. Acad. Sci. USA, 70, 2281-2285, 1973.  <sup>4</sup> Ames, B.N., McCann, J., and Yamasaki, E., "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364, 1975.  <sup>5</sup> See listing of codes, p.35.

**C. Chromosome Aberration Test in Human Lymphocytes In Vitro**

Test substance: 2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6

Method: Method B10 of Commission Directive 92/69/EEC and OECD Guidelines  
Method 473

Type: Genetic Toxicology: Chromosome Aberration Test

System of testing: Cultured human lymphocytes

GLP: Yes

Year: 2001

Results:

Cells/Cell Culture: Cells obtained from the peripheral circulation of suitable volunteers. The human lymphocytes were grown in supplemented Eagle's minimal essential medium (MEM) with HEPES buffer.

Test and Control Materials: The test material was dissolved in acetone and serially diluted. Final concentrations of the test material included 0, 51, 102, 204, 408, 612, and 816 µg/ml (Concentrations selected were based on results of a preliminary assay). Vehicle and positive controls were used in parallel with the test material. The vehicle control was acetone. The positive controls were mitomycin C dissolved in MEM at 0.4 and 0.2 µg/ml, in the absence of S9 and Cyclophosphamide dissolved in dimethyl sulphoxide at 12.5 µg/ml, in the presence of S9.

Treatment: Treated lymphocytes were evaluated for chromosomal aberrations at up to four dose levels, together with vehicle and positive controls. Four treatment conditions were used for the study. In Experiment 1, 4 hours in the presence of an induced rat liver homogenate metabolising system (S9), at a 1% final concentration with cell harvest after a 20-hour expression period, or a 4-hour exposure in the absence of metabolic activation (S9) with a 20-hour expression period. In Experiment 2, the 4-hour exposure with the addition of S9 was repeated (using a 2% final S9 concentration) whilst in the absence of metabolic activation the exposure time was increased to 24 hours.

Results: All vehicle (solvent) controls gave frequencies of cells with aberrations excluding gaps within the range expected for normal human lymphocytes. All positive control treatments gave statically significant increase in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolizing system. The test material did not induce any statistically significant increase in the frequency of cells with aberrations, in either of two separate experiments, using a dose range that included a dose level that induced approximately 50% mitotic inhibition. However, in the 4-hour exposure group without metabolic activation the limiting factor was the presence of test material precipitate where maximum exposure was achieved at 816 µg/ml.

Conclusion: The test material was shown to be non-clastogenic to human lymphocytes *in vitro* both in the presence or absence of S9.

Remarks:

This study is assigned a rating code of 1a<sup>2</sup>.

References:

<sup>1</sup>Chromosome Aberration Test in Human Lymphocytes In Vitro; SPL Project Number 971/120. Safepharma Laboratories Ltd, Derby, UK; 2001.

<sup>2</sup>See listing of codes, p.35.



## 15. REPEATED DOSE TOXICITY

### A. Subchronic (30 day) toxicity test in rats

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	<p>This study was conducted to determine the potential oral toxicity of the test article upon continuous administration in feed for 30 days to rats. The test material was fed at dietary levels of 0, 1.25, 2.5, or 5.0% to groups consisting of 10 males each.</p> <p>The body weight and food consumption of each rat was recorded, permitting mean food intake and weight gain to be determined. After 30 days, rats were killed and examined grossly.</p>
Species/strain:	Albino CF Nelson rats
Weight at initiation:	<u>Mean weight gain:</u> Control: 156 g 1.25%: 142 g 2.50%: 129 g (Significantly different from controls) 5.00%: 131 g (Significantly different from controls)
Sex:	Male
No. animals/group:	10 males / group
Route of administration:	Dietary
Exposure period:	30 days
Frequency of treatment:	Daily
Dose:	0, 1.25, 2.5, and 5% in food
GLP:	No
Year:	1965
Results:	<p>During the feeding period, animals at the 1.25% level were comparable to the controls, but dietary levels of 2.5% and 5.0% were not well tolerated. Animals at these 2 higher levels had a poor appearance. Some rats in each test group showed signs of gross hematuria, and one death occurred in the 5.0% group. At autopsy, intact kidneys of animals for the two high dose groups appeared normal, but cut sections revealed yellow masses in the renal tubules. Similar yellow masses were noted in the urinary bladders of several animals at these levels. The masses are believed to be glucuronides which have been observed with other benzophenone derivatives.</p>

Mean weight gain of the 2.5% and 5.0% groups, and mean food intake of the 2.5% feeding level, were significantly lower than corresponding

values for the controls. Mean daily dosage was calculated as 1.22 g/kg, 2.29 g/kg, and 4.78 g/kg for the 1.25%, 2.5% and 5.0% dietary levels, respectively.

The no-toxic-effect level (NOEL) was not determined.

Remarks:

This study is assigned a reliability code of 2e<sup>2</sup> (meets generally acceptable scientific standards, is well documented, and acceptable for assessment) according to the guidelines described by Klimisch *et al* (1997).

Reference:

<sup>1</sup>American Cyanamid Company. Subchronic (30 day) toxicity test in rats; Report No: 65-58, June 30, 1965.

<sup>2</sup>See listing of codes, p.35.

**B. Subchronic (90 day) toxicity test in rats**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone, 99.0% LOT # 8072 CAS No. 1843-05-6
Method:	This study was conducted to determine the potential oral toxicity of the test article upon continuous administration in feed for 3 months to rats. The test material was fed at dietary levels of 0, 0.2, 0.6, or 1.8% to groups consisting of 20 males and 20 females. The diet was prepared at weekly intervals and was available ad lib. The body weight of each rat was recorded twice a week. The food consumption of each group was determined weekly. Haematological data were collected from 5 animals of each sex animals in the 11 <sup>th</sup> week.. At the time of autopsy, liver and kidney weights were determined for 10 animals of each sex/group. 15 animals of each sex/group were examined grossly, weights of organs were recorded, and extensive histopathological examinations were conducted.
Species/strain:	Carworth Farms, Nelson strain albino rats, approximately 5 weeks of age at start of study.
Weight at initiation:	<u>Mean weight:</u> males: 77-125 g females: 75-110 g
Sex:	Male/Female
No. animals/group:	20 males & 20 females/ group
Route of administration:	Dietary
Exposure period:	90 days
Frequency of treatment:	Daily
Dose:	0, 0.2, 0.6 and 1.8% in feed
GLP:	Conducted prior to GLPs
Year:	1965
Results:	<p>The overall appearance and behaviour of all animals were good throughout the feeding period. Only 2 deaths occurred during the study period, a 0.2% female found dead on day 5 and a 0.6% male which lost weight in week 11 and died in week 12. Advanced autolysis prevented the determination of the cause of death in both instances. However, since no deaths occurred at the maximum feeding level of 1.8% it was concluded that the deaths were not related to the test article.</p> <p>There were no significant differences in mean weight gain between any test group and its corresponding control. There were no significant differences in mean food intake between test and control animals. There were no significant differences in mean hemoglobin or mean hematocrit between and of the test groups. Females in the 0.2% group had a very</p>

slight but significant decrease in total leukocyte, but was judged to be coincidental and unrelated to feeding of the test material as not differences in leukocyte counts were noted in the two higher dietary levels.

Mean liver weight of the 0.2% males and of both sexes at 0.6% was significantly increased, but no effect on liver weight was noted in the 1.8% dietary groups. Thus this was determined to be unrelated to the test article.

Mean kidney weight was significantly decreased for the 0.2% females and significantly increased for the 1.8% males. There was a slight, but not significant increase in the kidney weights of the 1.8% females. The observed increases in kidney weight at the 1.8% dietary levels could be test article related.

No significant lesions were encountered at the time of autopsy. The no-toxic-effect level (NOEL) was 0.6% in the diet.

Remarks:

This study is assigned a reliability code of 2e<sup>2</sup> (meets generally acceptable scientific standards, is well documented, and acceptable for assessment) according to the guidelines described by Klimisch *et al* (1997).

Reference:

<sup>1</sup>American Cyanamid Company. Sub-Chronic (90-day) toxicity test with UV-531 in rats. Report # 65-64. July 15, 1965.

<sup>2</sup>See listing of codes, p.35.

**C. Subchronic (90 day) toxicity test in rats**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6
Method:	This study was conducted to determine the potential oral toxicity of the test article upon continuous administration in feed for 3 months to rats. The test material was fed at dietary levels of 0, 0.065, 0.1, or 0.15% to groups consisting of 10 males and 10 females. The diet was prepared bi-weekly, and was constantly available. The body weight of each rat was recorded once a week. The food consumption of each group was determined in the first 4 weeks and in the 11 <sup>th</sup> and 12 <sup>th</sup> weeks. Haematological data were collected from all animals in the 13 <sup>th</sup> week, and biochemical data collected in the 14 <sup>th</sup> week. At 14 weeks, rats were sacrificed, examined grossly, and weights of organs were recorded. Extensive histopathological examinations were conducted.
Species/strain:	Wistar rats
Weight at initiation:	<u>Mean weight:</u> males: 41 - 62 g females: 43 - 61 g
Sex:	Male/Female
No. animals/group:	40 males & 40 females/ group
Route of administration:	Dietary
Exposure period:	90 days
Frequency of treatment:	Daily
Dose:	0, 0.065, 0.1 and 0.15% in food
GLP:	No
Year:	1968
Results:	<p>No deaths or untoward reactions were recorded throughout the 90-day feeding study. Gain in body weight, food intake, food efficiency, haematology, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase and alkaline phosphatase in the serum showed no treatment related abnormalities at any dietary level.</p> <p>Organ-to-body weight ratios were slightly increased for the kidney at the highest feeding level in females and for the thyroid at the two highest levels in males. Gross and microscopic examination did not reveal pathological changes attributed to the feeding.</p> <p>The no-toxic-effect level (NOEL) was 0.15% in the diet.</p>

- Remarks: This study is assigned a reliability code of 2e<sup>2</sup> (meets generally acceptable scientific standards, is well documented, and acceptable for assessment) according to the guidelines described by Klimisch *et al* (1997).
- Reference: <sup>1</sup>Sub-Chronic (90-day) toxicity test with “V 67-531” in rats. Report # 2769; Drs H.P. Til, Miss Drs. H.C. van der Meulen, Drs. J.W. Huismans and Dr. A.P. de Groot; November 1968; Central Institute for Nutrition and Food Research.
- <sup>2</sup>See listing of codes, p.35.

**D. Subchronic (90 day) toxicity test in rats**

Test substance:	2-Hydroxy-4-n-Octoxybenzophenone CAS No. 1843-05-6; Batch No. EN
Method:	This toxicity study was conducted to determine the potential oral toxicity of the test article upon continuous administration in feed for 3 months to rats. The diet for each test group was prepared weekly by blending the appropriate amount of the test compound with standard rat diet. Concentrations of the test compound in the feed were 1000 and 6000 ppm. Food and water were available ad libitum. Abnormal reactions and deaths were recorded daily. Body weights and food consumption were recorded weekly. Haematology, clinical blood chemistry, and urine were analyzed after 45 and 84 days. Surviving animals were sacrificed after 90 days and subject to gross examination. Organ weights were recorded and microscopic examination was conducted.
Species/strain:	Albino Rats, Charles River strain
Sex:	Male/Female
No. animals/group:	15 males & 15 females/ group
Route of administration:	Dietary
Exposure period:	90 days
Frequency of treatment:	Daily
Dose:	1,000 and 6,000 ppm
GLP:	No
Year:	1969
Results:	No outstanding differences in food consumption, body weight, or weight gain were observed between test and control rats. There were no effects of treatment on haematology, blood chemistry, or urinalysis. The liver and kidney weights were higher among males in the 6000 ppm group compared to controls. The gonad weights were higher among females in the 1000 ppm group relative to controls. There were no effects of treatment on gross pathology.
Remarks:	This study is assigned a reliability code of 2e <sup>2</sup> (meets generally acceptable scientific standards, is well documented, and acceptable for assessment) according to the guidelines described by Klimisch <i>et al</i> (1997)
Reference:	<sup>1</sup> Ninety-Day Subacute Oral Toxicity Of TU-1103 In Albino Rats; IBT No: B6680, January 8, 1969, Industrial Bio-test laboratories, Inc.  <sup>2</sup> See listing of codes, p.35.

**16. REPRODUCTIVE/DEVELOPMENTAL TOXICITY**

Test substance: 2-Hydroxy-4-n-Octoxybenzophenone  
CAS No. 1843-05-6  
99.0% Pure, WB-0785

Method: This toxicity study was conducted to determine whether ingestion of the test material would interfere with reproduction, lactation, or development of offspring. The reproduction and lactation performance, which was evaluated in terms of the criteria proposed by Oser and Oser (1959), is summarized below for each mating for the 4 generations combined. The study followed the general recommendations in "The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (The Association of Food and Drug Officials of the United States, pp 43, ff., (1959).

**Methods:**

Young adults, approximately 4-6 weeks of age were received in the laboratory and subsequently mated when they were ~3 months of age. This initial mating was conducted to ensure that the progenitors of the study were fecund. At 21 days of age, the offspring of the stock animals were placed on the study as the F<sub>0</sub> generation. The series of generations were managed according to the outline below:

F<sub>0</sub> Generation

⇒F<sub>1</sub>Generation

⇒F<sub>2</sub>Generation

⇒F<sub>3a</sub>Generation (Used for microscopic pathology and skeletal examination)

⇒F<sub>3b</sub>Generation

⇒F<sub>4</sub> Generation

**Diet:**

At weaning, the offspring produced by mating of the stock animals were placed on the study as the F<sub>0</sub> Generation. They were assigned to diets containing 0 (control) or 0.6% (6000 ppm) dietary concentration (~523.9 mg/kg/day (males) and ~614.3 mg/kg/day (females)). The test material was incorporated into ground lab chow on a weight basis, prepared weekly. Pups were weaned directly onto the diets their parents had been receiving.

**Management:**

Animals were housed singly after weaning; as pairs for mating. Food and water were available ad libitum. Abnormal reactions and deaths were recorded daily. Body weights were recorded periodically for all generations and food consumption was measured to time of mating for the F<sub>0</sub> Generation.

Species/strain: Albino Rats, Charles River CD strain

Sex: Male/Female

No. animals/group: 16 males & 16 females/ group; 80 matings cumulatively.



Route of administration:	Dietary
Exposure period:	4 Generations
Frequency of treatment:	Daily
Dose:	0 and (0.6%) 6,000 ppm
GLP:	No
Year:	1969

**Results:** The fertility, gestation, viability and lactation indices for the test and control groups were comparable and quite high in most instances. An occasional dip in one of the indices was followed by a higher value in a subsequent mating, thus indicating that there was no impairment of reproduction or lactation performance. Overall, there was an identical distribution of values for both groups. Consequently, it was concluded that a diet containing 0.6% (6000 ppm) of the test material had no adverse effect on the reproduction and lactation performance of rats.

There were no apparent differences in the number of live births or the number of pups weaned between the control and test groups. The average pup from rats fed the test material weighed slightly more than the average control pups.

Pups of all litters, including those which died before weaning, were examined for gross defects. However, autopsies were performed only on pups from the first mating of the F2 animals (F3aGeneratin). The latter pups were killed at weaning. Immediately after death, the 2 males and 2 females which were the smallest or least healthy appearing of each litter were set aside while the others were autopsied and examined for gross lesions. One of each sex of those set aside was autopsied, and portions of all organs were taken for histologic processing and examination. The other 2 animals were examined for skeletal defects using skeletal staining. The only condition which occurred frequently was hydronephrosis. This is a known spontaneous lesion in rats. Most cases were unilateral, involving the right kidney, and males were afflicted more frequently than females. The incidence of this condition was higher in control animals. Microscopic findings were few and clearly not related to treatment.

No skeletal abnormalities were seen in the control or treated pups. A group of 3 treated pups was sacrificed at 12 days of age after the mother had eaten their littermates. Their cleared skeleton appeared normal except for an apparent reduction in length of all bones, as compared to control animals of the same age. This may have resulted from maternal mineral deficiency.

It is concluded that feeding the test material to rats from weaning through reproductive age for 4 successive generations at a level of 0.6% did not produce lesions in the parents or anomalies in the offspring which could be attributed to the compound.

Remarks: This study is assigned a reliability code of 2e<sup>2</sup> (meets generally acceptable scientific standards, is well documented, and acceptable for assessment) according to the guidelines described by Klimisch *et al* (1997).

Reference: <sup>1</sup>Oser and Oser (1959) J. Nutrition, 60: 498-505.

<sup>2</sup>American Cyanamid Company. .Four Generation Reproduction and Lactation Study in Charles River Albino Rats; Report No: 69-251, December 18, 1969.

<sup>3</sup>See listing of codes, p.35.

**GENERAL REFERENCE**

Klimisch, H.J., Andreae, M and Tillman, U. *A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology.* 25: 1-5, 1997

1 = Valid without restriction

1a: GLP guideline study

1b: Comparable to guideline study

1c: Meets national standard methods (AFNOR/DIN)

1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction

2a: Guideline study without detailed documentation

2b: Guideline study with acceptance restrictions

2c: Comparable to guideline study with acceptable restrictions

2d: Meets national standard methods with acceptable restrictions

2e: Meets generally accepted scientific standards, well documented and acceptable for assessment

2f: Accepted calculation method

2g: data from Handbook or collection of data

3 = Invalid

3a: Documentation insufficient for assessment

3b: Significant methodological deficiencies

3c: Unsuitable test system

4 = Not assignable

4a: Abstract

4b: Secondary literature

4c: Original reference not yet available

4d: Original reference in a foreign language

4e: Documentation insufficient for assessment